#### AN INTERNATIONAL ORGANIZATION DEDICATED TO PROTECTING THE RIGHTS OF ALL ANIMALS

July 20, 2023

The Honorable Lloyd J. Austin III Secretary of Defense Department of Defense

The Honorable Carlos Del Toro Secretary of the Navy c/o Molly Papermaster, Special Assistant

Via e-mail: <a>ExecSecMA2@sd.mil</a>; <a href="mailto:molly.papermaster@navy.mil">molly.papermaster@navy.mil</a>

Dear Secretaries Austin and Del Toro:

Thank you in advance for your time. I'm writing on behalf of People for the Ethical Treatment of Animals—PETA entities have more than 9 million members and supporters globally—to follow up on our June 8, 2022, letter to Secretary Del Toro,<sup>1</sup> and our September 1, 2022, letter to Secretary Austin.<sup>2</sup> Based on the disturbing new information presented below, we urge you to ban the conducting and funding of the U.S. Navy's cruel decompression sickness/illness (DCS/DCI) and oxygen toxicity experiments on all animals and instead pursue animal-free, human-relevant research.

# The Navy Pulled Funding for Decompression Tests on Sheep at UW-Madison

PETA recently obtained public records showing that the gruesome decompression tests on sheep at the University of Wisconsin–Madison (UW-Madison) that the Navy had been funding and that had been awarded more than \$389,000 in taxpayer money have been abruptly stopped up to two years ahead of schedule.<sup>3</sup>

In one Navy-funded decompression experiment at UW-Madison initially approved from August 11, 2020, through August 10, 2023,<sup>4</sup> two healthy sheep were placed in a hyperbaric chamber for behavioral and equipment monitoring.



PETA

#### Washington

1536 16th St. N.W. Washington, DC 20036 202-483-PETA

#### Los Angeles

2154 W. Sunset Blvd. Los Angeles, CA 90026 323-644-PETA

# Norfolk

501 Front St. Norfolk, VA 23510 757-622-PETA

Info@peta.org PETA.org

#### Entities

- PETA Asia
- PETA India
- PETA France
- PETA Australia

- PETA Switzerland
- PETA Netherlands
- PETA Foundation (U.K.)

<sup>&</sup>lt;sup>1</sup>Pons S, Balsam MJ. Letter from PETA to the Honorable Carlos Del Toro. June 8, 2022. Accessed June 21, 2023. <u>https://www.peta.org/wp-content/uploads/2022/08/2022-06-08-letter-to-usn-re-dcs-tests.pdf</u>

<sup>&</sup>lt;sup>2</sup>Swaminathan S. Letter from PETA to the Honorable Lloyd J. Austin III. September 1, 2022. Accessed June 21, 2023. <u>https://www.peta.org/wp-content/uploads/2022/09/2022-09-01-letter-to-sec-of-defense-1.pdf</u>

<sup>&</sup>lt;sup>3</sup>Toropin K. Navy ends "gruesome" testing on sheep after PETA protests. Military.com. February 1, 2023. Accessed June 21, 2023. <u>https://www.military.com/daily-</u> news/2023/02/01/navy-ends-gruesome-testing-sheep-after-peta-protests.html

<sup>&</sup>lt;sup>4</sup>UW-Madison Institutional Animal Care and Use Committee Protocol Number V00: V006375. Accessed January 13, 2023. <u>https://www.peta.org/wp-content/uploads/2023/01/uw-madison-iacuc-V006375.pdf</u>

PETA Germany

A UW-Madison internal investigation found that on October 11, 2021, "the compressor for the chamber was not working" and that the animals inside the chamber had shown "signs of discomfort" and were then euthanized.<sup>5</sup> This experiment—and a separate Navy-funded decompression experiment at UW-Madison initially approved from June 2, 2021, through June 1, 2024, examining survival rates and cardiopulmonary function in sheep subjected to long-term exposure of hyperbaric air<sup>6</sup>—were *both* "terminated" on July 19, 2022, well ahead of schedule. This action followed PETA's June 8, 2023, letter to Secretary Del Toro urging an end to these tests.<sup>7</sup>

In 2010, the Navy also pulled its funding for similar decompression experiments on sheep at UW-Madison, following a criminal investigation launched in response to a petition filed by PETA and Alliance for Animals,<sup>8</sup> for which a court-appointed special prosecutor reported that "[t]he Department of the Navy has pulled its grant and the [decompression] research using sheep has stopped. In reviewing the more recent literature, it appears that the efficacy of these types of studies is now in question."<sup>9</sup>

# New Records Confirm Navy Funding DCS/DCI and Oxygen Toxicity Tests on Animals at Other Universities

PETA has recently obtained separate records confirming that the Navy is currently funding and/or has recently funded cruel and ineffective DCS/DCI and oxygen toxicity tests on other animals such as mice and rats at Duke University, the University of Maryland–Baltimore (UMB), the University of California–San Diego (UCSD), and the University of South Florida (USF).<sup>10</sup>

# Duke University

In an experiment conducted at Duke University titled "The Role of GABA in Susceptibility to Oxygen Toxicity With Repeated HBO2 Exposure"—approved by the school's Institutional Animal Care and Use Committee (IACUC) from November 19, 2021, to October 31, 2024,<sup>11</sup> and funded by the Office of Naval Research (ONR) from September 1, 2022, to August 31, 2025,

<sup>&</sup>lt;sup>5</sup>UW-Madison School of Veterinary Medicine Animal Care and Use Committee Closed Session [Minutes]— October 12, 2021. Accessed January 13, 2023. <u>https://www.peta.org/wp-content/uploads/2023/01/uw-madison-iacuc-closed-session.pdf</u>

<sup>&</sup>lt;sup>6</sup>UW-Madison IACUC Protocol Number V006315. Accessed January 13, 2023. <u>https://www.peta.org/wp-content/uploads/2023/01/uw-madison-iacuc-V006315.pdf</u>

<sup>&</sup>lt;sup>7</sup>Pons S, Balsam MJ. Letter from PETA to the Honorable Carlos Del Toro. June 8, 2022. Accessed June 21, 2023. <u>https://www.peta.org/wp-content/uploads/2022/08/2022-06-08-letter-to-usn-re-dcs-tests.pdf</u>

<sup>&</sup>lt;sup>8</sup>State of Wisconsin Circuit Court. Dane County. Brief in Support of Petition to Circuit Court Judge for Relief Pursuant to Wis. Stat. § 968.02(3). Injunction Relief 30704. March 16, 2010. Accessed January 13, 2023. https://www.peta.org/wp-content/uploads/2022/10/UW-sheep-decompression-brief-031610.pdf

<sup>&</sup>lt;sup>9</sup>State of Wisconsin Circuit Court. Dane County. Report and Determination by Special Prosecutor. Case Number 10CV1398. May 19, 2011. Accessed January 13, 2023. <u>https://www.peta.org/wp-content/uploads/2022/05/final-report-of-special-prosecutor.pdf</u>

<sup>&</sup>lt;sup>10</sup>U.S. Navy. Responsive Records. Decompression Sickness/Illness Oxygen Toxicity Research. April 18, 2023. Accessed June 21, 2023. <u>https://www.peta.org/wp-content/uploads/2023/06/2023-04-18-documents-obtained-final.pdf</u>

<sup>&</sup>lt;sup>11</sup>U.S. Navy. Responsive Records. Duke University. [p. 3]. Accessed June 10, 2023. <u>https://www.peta.org/wp-content/uploads/2023/06/navy-responsive-records-duke-university.pdf</u>

with \$844,714 in taxpayer money<sup>12</sup>—to determine how hyperbaric oxygen and GABAergic neurotransmission influence repeated exposure sensitivity to oxidative tissue injury, experimenter Heath Gasier induces seizures in up to 592<sup>13</sup> juvenile mice. The animals are injected with an experimental agent, exposed to high-pressure oxygen at 5 ATA in hyperbaric chambers for 60 minutes at varying intervals of up to 72 hours, and are forced to run on a treadmill with a shock grid on which they are subjected to shocks at 0.2 mA if they fail to keep up with the pace of the treadmill for five seconds. This level of stimulus is highly aversive to rats.<sup>14</sup> The animals are also restrained by holding them in place on their backs to collect blood from their tails and ultimately killed by exposure to isoflurane gas.<sup>15</sup>

## UMB

In an experiment conducted at UMB titled "Neuroinflammation and Glymphatic Dysfunction in Decompression Sickness"—approved by the UMB IACUC from March 11, 2022, to February 18, 2025,<sup>16</sup> and funded by ONR from June 1, 2022, to May 31, 2025, with \$981,996.45 in taxpayer money<sup>17</sup>—to evaluate the neuroinflammatory impact of high pressure and decompression, experimenter Stephen Thom subjects as many as 2,180 mice to some of the following procedures: injection of one of several experimental agents via a tail vein catheter as well as one of two types of contrast media via a tail vein catheter or into the cerebrospinal fluid through a catheter placed in the occipital region of the skull<sup>18</sup>; exposure to up to 100 psi air for two hours followed by excruciatingly painful decompression<sup>19</sup>; injection with inflammatory microparticles obtained from cervical nodes of previously decompressed and killed mice<sup>20</sup>; survival surgery to remove cervical nodes, followed by exposure to 100 psi air for two hours and subsequent decompression<sup>21</sup>; and post-decompression behavioral testing, for instance by forcing animals to navigate an accelerated roto-rod.<sup>22</sup> Animals used in this experiment are then killed by exposure to ketamine/xylazine, and their blood and tissues are collected for analysis.<sup>23</sup>

<sup>15</sup>U.S. Navy. Responsive Records. Duke University. [p. 59]. Accessed June 27, 2023.

https://www.peta.org/wp-content/uploads/2023/06/navy-responsive-records-duke-university.pdf

<sup>&</sup>lt;sup>12</sup>Defense Technical Information Center. Award Number: N000142212749. The role of GABA in susceptibility to oxygen toxicity with repeated HBO2 exposure. Accessed June 8, 2023. https://publicaccess.dtic.mil/search/#/grants/simpleSearch

<sup>&</sup>lt;sup>13</sup>U.S. Navy. Responsive Records. Decompression Sickness/Illness Oxygen Toxicity Research. April 18, 2023. [p. 46] Accessed June 26, 2023. <u>https://www.peta.org/wp-content/uploads/2023/06/2023-04-18-documents-obtained-final.pdf</u>

<sup>&</sup>lt;sup>14</sup>Lichtenberg NT, Lee B, Kashtelyan V, Chappa BS, Girma HT, Green EA, Kantor S, Lagowala DA, Myers MA, Potemri D, Pecukonis MG, Tesfay RT, Walters MS, Zhao AC, Blair RJR, Cheer JF, Roesch MR. Rat behavior and dopamine release are modulated by conspecific distress. (2018). eLife. **7**:e38090.

 <sup>&</sup>lt;sup>16</sup>U.S. Navy. Responsive Records. Decompression Sickness/Illness Oxygen Toxicity Research. April 18, 2023. [p. 7]. Accessed June 21, 2023. <u>https://www.peta.org/wp-content/uploads/2023/06/2023-04-18-documents-obtained-final.pdf</u>

<sup>&</sup>lt;sup>17</sup>Defense Technical Information Center. Award Number: N000142012434. Mitigation of CNS oxygen toxicity: Mitochondria targeted anti-oxidant therapy and aquaporin gas channels. Accessed May 10, 2023. https://publicaccess.dtic.mil/search/#/grants/simpleSearch

<sup>&</sup>lt;sup>18</sup>U.S. Navy. Responsive Records. Decompression Sickness/Illness Oxygen Toxicity Research. April 18, 2023. [p. 20]. Accessed June 21, 2023. <u>https://www.peta.org/wp-content/uploads/2023/06/2023-04-18-documents-obtained-final.pdf</u>

<sup>&</sup>lt;sup>19</sup>*Ibid*. [pp. 20, 21, 23, and 27]

<sup>&</sup>lt;sup>20</sup>*Ibid.* [p. 24]

<sup>&</sup>lt;sup>21</sup>*Ibid.* [p. 27]

<sup>&</sup>lt;sup>22</sup>*Ibid.* [p. 29]

<sup>&</sup>lt;sup>23</sup>*Ibid.* [p. 33]

## UCSD

In an experiment conducted at UCSD titled "Nitrogen Gas Uptake and Excretion During Compression/Decompression to Simulate Diving"—approved by the UCSD IACUC from November 11, 2019, to November 26, 2022,<sup>24</sup> and funded by ONR under the title "Where Is the Gas? A New Method to Study Nitrogen During Diving and Decompression: FY2019-000313-AS" from August 1, 2020, to July 31, 2023, with \$1,009,127 in taxpayer money<sup>25</sup>—to develop a tool for direct mapping of nitrogen gas tissue distribution when in a hyperbaric environment, experimenter Peter Lindholm forces as many as 50 rats to "breathe radioactive gas while compressed in a pressure chamber" at 700 kPa (equivalent to 100 psi) for up to 45 minutes.<sup>26</sup> Prior to compression and decompression experiments, some of the animals are forced to exercise for 90 minutes on a commercially available treadmill with the application of highly aversive<sup>27</sup> shocks (at 2mA for five consecutive seconds) if the rats do not run willingly.<sup>28</sup> At the end of the experiments, the animals are killed by exposure to isoflurane.<sup>29</sup>

# USF

In an experiment conducted at USF titled "Mitigation of CNS Oxygen Toxicity: Mitochondria Targeted Anti-Oxidant Therapy and Aquaporin Gas Channels"—approved by the USF IACUC from December 6, 2022, to December 6, 2025,<sup>30</sup> and funded by ONR from April 16, 2020, to April 15, 2023, with \$1,004,575 in taxpayer money<sup>31</sup> as well as in another Navy-funded project<sup>32</sup>—to test if electrodermal activity increases during the pre-seizure period of central nervous system oxygen toxicity (CNS-TN) induced by breathing hyperbaric oxygen, experimenter Jay Dean subjects as many as 370 animals<sup>33</sup> to high pressure (5 ATA hyperbaric

<sup>&</sup>lt;sup>24</sup>U.S. Navy. Responsive Records. Decompression Sickness/Illness Oxygen Toxicity Research. March 6, 2023. [p. 117]. Accessed June 21, 2023. <u>https://www.peta.org/wp-content/uploads/2023/06/2023-04-18-documents-obtained-final.pdf</u>

<sup>&</sup>lt;sup>25</sup>Defense Technical Information Center. Award Number: N000142012763. Where is the gas? A new method to study nitrogen during diving and decompression: FY2019-000313-AS. Accessed June 27, 2023. https://publicaccess.dtic.mil/search/#/grants/simpleSearch

<sup>&</sup>lt;sup>26</sup>U.S. Navy. Responsive Records. Decompression Sickness/Illness Oxygen Toxicity Research. March 6, 2023. [p. 270]. Accessed June 21, 2023. <u>https://www.peta.org/wp-content/uploads/2023/06/2023-04-18-documents-obtained-final.pdf</u>

<sup>&</sup>lt;sup>27</sup>Lichtenberg NT, Lee B, Kashtelyan V, Chappa BS, Girma HT, Green EA, Kantor S, Lagowala DA, Myers MA, Potemri D, Pecukonis MG, Tesfay RT, Walters MS, Zhao AC, Blair RJR, Cheer JF, Roesch MR. Rat behavior and dopamine release are modulated by conspecific distress. (2018). eLife. **7**:e38090.

<sup>&</sup>lt;sup>28</sup>U.S. Navy. Responsive Records. Decompression Sickness/Illness Oxygen Toxicity Research. March 6, 2023. [p. 190]. Accessed June 21, 2023. <u>https://www.peta.org/wp-content/uploads/2023/06/2023-04-18-documents-obtained-final.pdf</u>

<sup>&</sup>lt;sup>29</sup> *Ibid.* [p. 302]

<sup>&</sup>lt;sup>30</sup>U.S. Navy. Responsive Records. USF. Accessed April 18, 2023. <u>https://www.peta.org/wp-content/uploads/2023/04/navy-responsive-records-usf.pdf</u>

<sup>&</sup>lt;sup>31</sup>Defense Technical Information Center. Award Number: N000142012434. Mitigation of CNS oxygen toxicity: Mitochondria targeted anti-oxidant therapy and aquaporin gas channels. Accessed May 10, 2023. https://publicaccess.dtic.mil/search/#/grants/simpleSearch

<sup>&</sup>lt;sup>32</sup>Posada-Quintero HF, Landon CS, Stavitzski NM, Dean JB, Chon KH. Seizures caused by exposure to hyperbaric oxygen in rats can be predicted by early changes in electrodermal activity. *Front. Physiol.* 2022;12:767386. Published January 5, 2022.

<sup>&</sup>lt;sup>33</sup>U.S. Navy. Responsive Records. Decompression Sickness/Illness Oxygen Toxicity Research. March 6, 2023. [p. 2]. Accessed June 21, 2023. <u>https://www.peta.org/wp-content/uploads/2023/06/2023-04-18-documents-obtained-final.pdf</u>

oxygen) until the onset of seizures, without administering analgesics. He then cuts open their abdomens and embeds a radio telemetry device in them, places four wires traversing along each animal's back and neck to reappear at the skin over their skulls, restrains the animals while drilling into their skulls to place electrodes on them, and kills the animals after the experiments end.<sup>34,35</sup> Animals presenting with post-surgical complications, such as "abnormal physiology or behavior," who are unable to undergo subsequent exposure to hyperbaric air experiments, will be "removed and euthanized with  $CO_2$ "<sup>36</sup>—a particularly painful mode of killing, exacerbated by the post-surgical complications endured by the animals.<sup>37</sup>

In a separate but similar experiment conducted at USF titled "Electrodermal Activity During Hyperbaric Oxygen Exposure: Rat Study"—funded by ONR from February 1, 2019, to January 31, 2020, with \$24,977 in taxpayer money<sup>38</sup>—Dean implanted a radio telemetry device in rats' abdomens, implanted wire leads through the animals' skulls, and connected electrodes to the animals' skin to measure electrodermal activity during the onset of central nervous system oxygen toxicity seizures caused by subjecting the animals to high pressure (5 ATA hyperbaric oxygen).<sup>39</sup>

## Poor Data Translation From DCS/DCI Animal Testing to Humans

The management of detrimental effects from DCS/DCI or oxygen toxicity in humans (and other diving and nondiving animals)—ranging from slight or severe pain to paralysis or even death— are well established.<sup>40,41,42</sup> While the mechanistic triggers of these symptoms are still active areas of research, using animals in experiments as models for DCS/DCI or oxygen toxicity in humans is inhumane and ineffective. Meaningful evaluation of gas diffusion dynamics in live animals— and subsequent translation of this data to humans—is inaccurate due to the inherent complexity of the system under study, in which even the most subtle movements, such as breathing, can and too often do produce spurious results.<sup>43</sup>

<sup>&</sup>lt;sup>34</sup>U.S. Navy. Responsive Records. USF. [pp. 8 and 9]. Accessed April 18, 2023. <u>https://www.peta.org/wp-content/uploads/2023/04/navy-responsive-records-usf.pdf</u>

<sup>&</sup>lt;sup>35</sup>Posada-Quintero HF, Landon CS, Stavitzski NM, Dean JB, Chon KH. Seizures caused by exposure to hyperbaric oxygen in rats can be predicted by early changes in electrodermal activity. *Front. Physiol.* 2022;12:767386. Published January 5, 2022.

<sup>&</sup>lt;sup>36</sup>U.S. Navy. Responsive Records. University of South Florida. [p. 10]. Accessed April 18, 2023. https://www.peta.org/wp-content/uploads/2023/04/navy-responsive-records-usf.pdf

<sup>&</sup>lt;sup>37</sup>Conlee KM, Stephens ML, Rowan AN, King LA. Carbon dioxide for euthanasia: concerns regarding pain and distress, with special reference to mice and rats. *Lab. Anim.* 2005. 39(2):137-61.

<sup>&</sup>lt;sup>38</sup>U.S. Navy. Responsive Records. Decompression Sickness/Illness Oxygen Toxicity Research. March 6, 2023. [p. 328]. Accessed June 21, 2023. <u>https://www.peta.org/wp-content/uploads/2023/06/2023-04-18-documents-obtained-final.pdf</u>

<sup>&</sup>lt;sup>39</sup>U.S. Navy. Responsive Records. Decompression Sickness/Illness Oxygen Toxicity Research. March 6, 2023. [pp. 350–354]. Accessed June 21, 2023. <u>https://www.peta.org/wp-content/uploads/2023/06/2023-04-18-documents-obtained-final.pdf</u>

 <sup>&</sup>lt;sup>40</sup>Pollock NW, Buteau D. Updates in decompression illness. *Emerg. Med. Clin. North Am.* 2017;35(2):301-319.
<sup>41</sup>Tal D, Shachar-Bener H, Hershkovitz D, Arieli Y, Shupak A. Evidence for the initiation of decompression sickness by exposure to intense underwater sound. *J. Neurophysiol.* 2015;114(3):1521-1529.

<sup>&</sup>lt;sup>42</sup>Cronin WA, Khan K, Hall AA, Bodo M, Mahon RT. The effect of the perfluorocarbon emulsion Oxycyte<sup>™</sup> in an

ovine model of severe decompression illness. Undersea Hyperb. Med. 2021;48(1):25-31.

<sup>&</sup>lt;sup>43</sup>Walsh C, Stride E, Cheema U, Ovenden N. A combined three-dimensional *in vitro–in silico* approach to modelling bubble dynamics in decompression sickness. *J. R. Soc. Interface*. 2017;14(137):20170653.

In his May 12, 2010, reply to PETA's March 30, 2010, complaint regarding using animals in DSC/DCI testing to then Secretary of the Navy Ray Mabus, the then Director of the Navy Medical Research and Development Center W.W. Cheatham, M.D., admitted, "The impact of physiological differences between species with regard to disease processes, to include diving related issues, is well recognized throughout the medical research community."<sup>44</sup> Numerous experts have noted the inherent limitations of using animals to predict the effects of DCS/DCI in humans. Diving expert John Lippmann has stated, "The problem with these animal experiments is that no animal model can replicate what happens in a human."<sup>45</sup>

The Naval Medical Research Command itself has stated, "[A]nimal DCS in many cases is more severe than that in humans and, therefore, appears 'different' from the average human case. ... Among species, there certainly are differences in tolerance to decompression, with relative susceptibility to DCS tending to increase with species size. ... These observations suggest that response differences among species to the insult of decompression may reflect a combination of factors, including differences in gas exchange and tolerance to excess gas in the body."<sup>46</sup>

## Superior, Non-Animal DCS/DCI Research Methods Are Widely Available

Thankfully, modern, non-animal technology is available for studying DCS/DCI based on human tissue and data obtained from human divers.<sup>47,48,49,50,51,52</sup> Some options include the following:

• *In vitro* studies of human endothelial cells—later confirmed by human trials—provide researchers with evidence in simulated diving conditions of nitric oxide dynamics, a chemical compound that can protect against bubble formation.<sup>53,54,55</sup>

<sup>&</sup>lt;sup>44</sup>Cheatham, WW. Letter to Shalin G. Gala. PETA.org. Published May 3, 2022. Accessed May 3, 2022. <u>https://www.peta.org/wp- content/uploads/2022/05/letter-to-navy-reply.pdf</u>

<sup>&</sup>lt;sup>45</sup>Lippmann J. *The Essentials of Deeper Sport Diving: An Overview of the Theory and Requirements of Deeper Diving.* Locust Valley, NY: Aqua Quest Publications; 1992.

 <sup>&</sup>lt;sup>46</sup>Lillo RS, Himm JF, Weathersby PK, Temple DJ, Gault KA, Dromsky DM. Using animal data to improve prediction of human decompression risk following air-saturation dives. *J. Appl. Physiol.* 2002;93(1):216-226.
<sup>47</sup>Palkovic M, Novomeský F, Zaviacic M, Danihel L, Kovác P, Danisovic L. The use of cell cultures for in vitro decompression sickness simulation, *Soud. Lek.* 2007;52(1): 9-16.

<sup>&</sup>lt;sup>48</sup>Jang DH, Owiredu S, Ranganathan A, Eckmann DM. Acute decompression following simulated dive conditions alters mitochondrial respiration and motility. *Am. J. Physiol. Cell. Physiol.* 2018;315(5):C699-C705.

<sup>&</sup>lt;sup>49</sup>Wienke BR. Computer validation and statistical correlations of a modern decompression diving algorithm. *Comput. Biol. Med.* 2010;40(3):252-260.

<sup>&</sup>lt;sup>50</sup>Germonpré P, Lafère P, Portier W, Germonpré FL, Marroni A, Balestra C. Increased risk of decompression sickness when diving with a right-to-left shunt: Results of a prospective single-blinded observational study (the "Carotid Doppler" study). *Front. Physiol.* 2021;12:763408. Published October 29, 2021.

<sup>&</sup>lt;sup>51</sup>Honěk J, Šrámek M, Honěk T, et al. Screening and risk stratification strategy reduced decompression sickness occurrence in divers with patent foramen ovale. *JACC Cardiovasc. Imaging.* 2022;15(2):181-189.

<sup>&</sup>lt;sup>52</sup>Han KH, Hyun GS, Jee YS, Park JM. Effect of water amount intake before scuba diving on the risk of decompression sickness. *Int. J. Environ. Res. Public Health.* 2021;18(14):7601.

<sup>&</sup>lt;sup>53</sup>Hesthammer R, Eide T, Thorsen E, Svardal AM, Djurhuus R. Decrease of tetrahydrobiopterin and NO generation in endothelial cells exposed to simulated diving. *Undersea Hyperb. Med.* 2019;46(2):159-169.

<sup>&</sup>lt;sup>54</sup>Hesthammer R, Eide T, Thorsen E, Svardal AM, Djurhuus R. Hyperoxia and lack of ascorbic acid deplete tetrahydrobiopterin without affecting NO generation in endothelial cells. *Undersea Hyperb. Med.* 2019;46(4):509-519.

<sup>&</sup>lt;sup>55</sup>Hesthammer R, Dahle S, Storesund JP, Eide T, Djurhuus R, Svardal AM, Thorsen E. Nitric oxide in exhaled gas and tetrahydrobiopterin in plasma after exposure to hyperoxia. *Undersea Hyperb. Med.* 2020;47(2):197-202.

- Reanalysis of existing human diver data has led to improved models that may be better able to predict DCS/DCI symptoms and risk factors.<sup>56,57</sup>
- Machine-learning techniques may aid in the prediction of symptoms such as seizures during hyperbaric oxygen therapy.<sup>58</sup>
- Diver staging models can also improve the performance of dive computers to better equip divers in avoiding DCS/DCI,<sup>59</sup> and laboratory studies using human subjects are yielding novel data.<sup>60,61</sup>
- Recent technological advances have also facilitated the development of a combined "biomimetic *in vitro* tissue phantom and a three-dimensional computational model, comprising a hyperplastic strain-energy density function to model tissue elasticity" as well as investigating key areas of gas diffusion dynamics and developing more accurate dive algorithms.<sup>62</sup>

# The Navy's DCS/DCI Experiments on Animals Are out of Step With International Standards

Both the British and French navies have already ended their respective DCS animal-testing programs.<sup>63,64</sup> In 2008, then–U.K. Parliamentary Under Secretary of State at the Ministry of Defence Derek Twigg wrote to the British Parliament, stating, "The Ministry of Defence (MOD) has today announced the end of its immediate requirement for testing on live goats as part of its hyperbaric research in support of the MOD's Submarine Escape Rescue and Abandonment System (SMERAS)."<sup>65</sup> He described the findings motivating this decision in the following statement:

The testing programme was aimed at improving the accuracy of the information relating to the likely probability and consequence of decompression illness following escape from a submerged submarine in varying depths and internal submarine pressures. This requirement has now been achieved, and the review has concluded that the remaining associated areas of uncertainty in submarine escape and rescue relate to events that are considered highly unlikely, and do not therefore need to be addressed by means of animal testing. The MOD has

<sup>&</sup>lt;sup>56</sup>King AE, Andriano NR, Howle LE. Trinomial decompression sickness model using full, marginal, and non-event outcomes. *Comput. Biol. Med.* 2020;118:103640.

<sup>&</sup>lt;sup>57</sup>Cialoni D, Pieri M, Balestra C, Marroni A. Dive risk factors, gas bubble formation, and decompression illness in recreational SCUBA diving: Analysis of DAN Europe DSL data base. *Front. Psychol.* 2017;8.

<sup>&</sup>lt;sup>58</sup>D'Angelo G, Pilla R, Dean JB, Rampone S. Toward a soft computing-based correlation between oxygen toxicity seizures and hyperoxic hyperpnea. *Soft. Comput.* 2018;22(7):2421-2427.

<sup>&</sup>lt;sup>59</sup>Wienke BR, O'Leary TR. On bubble regeneration and broadening with implications for decompression protocols. *Significances Bioeng. Biosci.* 2018;2(4).

<sup>&</sup>lt;sup>60</sup>Hess HW, Hostler D, Clemency BM, St. James E, Johnson BD. Carotid body chemosensitivity is not attenuated during cold water diving. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 2021;321(2):R197-R207.

<sup>&</sup>lt;sup>61</sup>Hess HW, Hostler D, Clemency BM, Johnson BD. Carotid body chemosensitivity at 1.6 ATA breathing air versus 100% oxygen. *J. Appl. Physiol.* 2020;129(2):247-256.

<sup>&</sup>lt;sup>62</sup>Walsh C, Stride E, Cheema U, Ovenden N. A combined three-dimensional *in vitro–in silico* approach to modelling bubble dynamics in decompression sickness. *J. R. Soc. Interface*. 2017;14(137):20170653.

<sup>&</sup>lt;sup>63</sup>Norton-Taylor R. Navy used goats in submarine experiments, MoD reveals. *The Guardian*. February 6, 2008. Accessed May 26, 2023. <u>https://www.theguardian.com/uk/2008/feb/07/animalwelfare military</u>

<sup>&</sup>lt;sup>64</sup>BBC News. UK Navy to end goat experiments. February 6, 2008. Accessed May 26, 2023. http://news.bbc.co.uk/1/hi/7230867.stm

<sup>&</sup>lt;sup>65</sup>Hansard House of Commons. Written Ministerial Statements. February 6, 2008. Accessed May 26, 2023. <u>https://publications.parliament.uk/pa/cm200708/cmhansrd/cm080206/wmstext/80206m0002.htm</u>

endorsed these recommendations and as a result, it has no immediate need to continue animal testing of this type. $^{66}$ 

# The Navy's DCS/DCI Testing on Animals Appears to Contravene Federal Standards

The use of animals in DCS/DCI and oxygen toxicity testing is at variance with the existing regulations to minimize animal use in experiments. The U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training (1985) states, "The animals selected for a procedure should be of an appropriate species and quality and *the minimum number required to obtain valid results*" [*emphasis added*].<sup>67</sup> Given the widespread availability of non-animal, human-focused testing methods that can be used instead, the number of animals used for DCS/DCI and oxygen toxicity testing should be zero.

Furthermore, Defense Health Agency-Multi-Service Regulation (DHA-MSR) 6025.02 states, "Alternatives to animal use will be considered *and used* whenever possible and appropriate to attain the objectives of [Research, Development, Test, and Evaluation] or training if such methods produce scientifically or educationally valid or equivalent results" [*emphasis added*].<sup>68</sup> It's clear from the aforementioned precedents set at UW-Madison and by the British and French navies that there are valid alternatives to using animals for DCS/DCI and oxygen toxicity testing, and per this military regulation, such animal-free experimental methods should be used in place of experiments on animals.

## **Request for Action**

There is no scientific, legal, or ethical justification for subjecting animals to DCS/DCI or oxygen toxicity experiments. Therefore, we urge you to immediately ban all DCS/DCI and oxygen toxicity experiments on animals conducted and/or funded by the U.S. Navy. Shifting to cutting-edge, human-relevant research methods would be directly applicable to the welfare of military personnel and, ultimately, the public.

You can contact me at <u>MaggieW@peta.org</u>. Thank you for your consideration of this important issue. I look forward to your reply.

Sincerely yours,

Winieus

Maggie Wisniewska, Ph.D. Science Policy Advisor International Laboratory Methods Division Laboratory Investigations Department

<sup>66</sup>Ibid.

<sup>&</sup>lt;sup>67</sup>National Institutes of Health. National Center for Biotechnology Information. National Research Council Committee for the Update of the Guide for the Care and Use of Laboratory Animals. Appendix B: U.S. government principles for the utilization and care of vertebrate animals used in testing, research, and training. Accessed June 27, 2023. <u>https://www.ncbi.nlm.nih.gov/books/NBK54048/</u>

<sup>&</sup>lt;sup>68</sup>Cheatham, WW. Letter to Shalin G. Gala. PETA.org. Published May 3, 2022. Accessed May 3, 2022. <u>https://www.health.mil/-/media/Files/MHS/Policy-Files/DHAMSR-602502-The-Care-and-Use-of-Animals-in-DoD-Research.ashx</u>

 cc: The Honorable Erik K. Raven, Under Secretary of the Navy (<u>erik.raven@navy.mil</u>) Capt. Jereal Dorsey, Spokesperson, Office of the Secretary of the Navy (jereal.e.dorsey.mil@us.navy.mil) DoD Office of HSD Animal Protections Programs (<u>osd.re.rt.cto@mail.mil</u>) The Honorable Heidi Shyu, Under Secretary of Defense for Research and Engineering (<u>Heidi.Shyu.civ@mail.mil</u>; <u>osd.pentagon.ousd-re.mbx.communications@mail.mil</u>) Kevin Doxey, Executive Director, Defense Science Board (<u>kevin.a.doxey.civ@mail.mil</u>) The Honorable Rear Adm. Bruce L. Gillingham, Chief, Bureau of Medicine and Surgery (Bruce.L.Gillingham.mil@mail.mil)